UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION Unclassified	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distributed limited
AD-A224 873	5 MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR-TR- 90 0752
6a NAME OF PERFORMING ORGANIZATION Hahnemann University 6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION Air Force Office of Scientific Research/NL
6c ADDRESS (City, State and ZIP Code) Broad and Vine Philadelphia, PA 19102	7b ADDRESS (City, State and ZIP Code) Building 410 Bolling AFB, DC 20332-6448
8a NAME OF FUNDING/SPONSORING ORGANIZATION AFOSR 8b OFFICE SYMBOL (If applicable) NL	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER AFOSR-87-0138
8c ADDRESS (City, State and ZIP Code) Building 410 Bolling AFB, DC 20332	10 SOURCE OF FUNDING NOS PROGRAM ELEMENT NO: 61102F; PROJECT NO: 2312; TASK NO: A2; WORK UNIT NO:
11 TITLE (Include Security Classification) (See attached page)	
12 PERSONAL AUTHOR(S) Barry D. Waterhouse	
13a TYPE OF REPORT Annual Scientific Report 13b TIME COVERED FROM 04-01-89 TO 03-31-90	14 DATE OF REPORT (Yr, Mo, Day) 90-06-01 15 PAGE COUNT
16 SUPPLEMENTARY NOTATION	
17 COSATI CODES: FIELD / GROUP / SUB GR	18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Monoamines, norepinephrine (NE), serotonin (5-HT), selective attention, cerebellum, somatosensory cortex, visual cortex

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The work described here is part of an ongoing set of studies aimed at characterizing the physiological actions and anatomical organization of the monoaminergic projection systems to the rat cerebral cortex. The underlying theme of this work is that the endogenous monoamines, norepinephrine (NE) and serotonin (5-HT), serve to modulate central neuronal responsiveness to afferent synaptic inputs and by so doing participate in the cognitive process of selective attention. Individual studies conducted during the past year have investigated: 1.) the adrenergic and amino acid receptor specificity of NE-induced facilitation of glutamate efficacy, 2.) the influence of NE on GABA-induced membrane conductance changes in identified cortical neurons, 3.) the effects of NE on the receptive field properties of visual cortical neurons and 4.) the anatomical distribution of monoamine-containing cells that project via axon collaterals to multiple sites along the central somatosensory pathway. Overall, the data provide further support for the contention that the diffusely distributed monoamine systems of the mammalian brain may enhance the performance of target neuronal circuits as a function of changing behavioral conditions.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS ☐	21 ABSTRACT SECURITY CLASSIFICATION
22a NAME OF RESPONSIBLE INDIVIDUAL Dr William Berry	22b TELEPHONE NUMBER (Include Area Code) (202) 767-5021 22c OFFICE SYMBOL NL

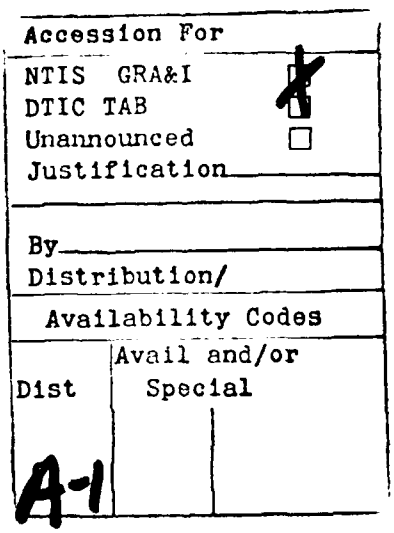
DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE

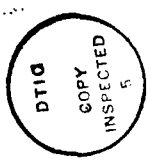
SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

Item #11.

The Role of Central Monoaminergic Systems in Arousal and Selective Attention





ANNUAL TECHNICAL REPORT

AFOSR-87-0138
"The Role of Central Monoaminergic Systems
in Arousal and Selective Attention."
4-1-89 to 3-31-90

Barry B. Waterhouse, Ph.D.
Department of Physiology and Biophysics
Hahnemann University
Broad and Vine
Philadelphia, PA 19102

1. Summary

A number of experimental approaches including intra- and extracellular recording of cells from cortical tissue slices, unit recording in awake behaving rats, extracellular recording from anesthetized, paralyzed cats and double retrograde labeling studies have been employed to further investigate the anatomical organization and physiological actions of the cortically projecting monoaminergic systems.

One aim of studies conducted during the past year was to further investigate the pharmacological specificity of norepinephrine (NE)- mediated facilitation of glutamate-induced cortical neuronal responses. Other studies conducted during the past year examined the effects of NE and related adrenergic agents on GABA-induced membrane conductance changes. These latter studies on cerebrocortical neurons were designed to identify the transmembrane events associated with previously observed GABA potentiating actions of extracellularly administered NE. The added significance of these intracellular experiments was that cortical neurons sensitive to noradrenergic modulatory actions could be identified according to electrophysiological and morphological criteria.

Other pilot studies in cat visual cortex began to investigate the effects of iontophoretically applied NE on specific receptive field properties (direction selectivity, orientation and velocity tuning) of individual area 17 neurons. A new anatomical study using double retrograde labeling strategies has been initiated in order to determine the distribution of brainstem noradrenergic and serotonergic neurons that send axon collaterals to multiple relay stations along the rat somatosensory pathway.

Finally, two reports detailing the effects of cocaine in monoaminergic target circuits of rat brain have been prepared for publication during the past year.

Overall, the studies conducted during the past year continue to contribute basic information concerning the potential modulatory role of NE and serotonin in monoaminergic target circuits of the mammalian brain.

2. Research Objectives

The primary focus of the project has not deviated from the originally stated aims listed below; with the exception that considerable effort is now being directed toward identifying the transmembrane effects of NE on cortical neurons via intracellular recording studies. The goal of these latter studies is to elucidate the mechanisms through which NE may express its neuromodulatory actions on sensory signals processed by cerebrocortical circuits.

Aim 1. Investigate the basic physiological actions of the noradrenergic and serotonergic projection systems in primary sensory areas of the mammalian brain. Initial studies will employ iontophoretic methods of drug application to characterize the elemental effects of NE and 5-HT on somatosensory or visual cortical neuronal responses to peripheral stimulation of afferent synaptic pathways or to microtiontophoresis of putative transmitter substances. In other studies, stimulation of the locus coeruleus or dorsal raphe nucleus will be employed to cause release of endogenous monoamines at anatomically relevant sites in target neuronal circuits and confirm results observed with iontophoretic application of NE and 5-HT. Similar studies will be carried out in other regions of the brain that relay sensory information and receive monoaminergic projections, e.g. lateral geniculate nucleus, superior colliculus. The goal here will be to further develop the concept that NE and 5-HT operate in a neuromodulatory mode as part of a signal "gating" or "filtering" mechanism in primary sensory areas of the neocortex and other sensory information relay circuits of the brain. While these initial studies will be carried out in anesthetized rats, a major effort will be mounted to examine these issues in awake, behaving animals using recently developed techniques for chronic unit recording.

Aim 2. Analysis of the physiological actions of amphetamine and cocaine at the synaptic level in cerebrocortical and cerebellar circuits. The primary issues to be investigated here are whether these psychostimulant agents can mimic the facilitating actions of NE on neuronal responsiveness to synaptic inputs and putative transmitter substances and whether or not such effects correlate with the overt behavioral responses which have been reported for these drugs. The proposed experiments will employ the electrophysilogical assays developed previously for study of the NE system to determine the effects of these drugs on synaptic mechanisms. The merit of this approach derives from the fact that a common set of experimental neurophysiological tests can be used to examine the action of compounds which are known to interact with this system.

Aim 3. Examine the anatomical organization of monoamine-containing projection neurons with respect to sensory-specific target regions of the CNS. These investigations will employ single and double retrograde tracer techniques to study the distribution of monoamine-containing projection neurons with respect to sensory-modality specific target regions in the CNS. Initial studies using retrograde transport of HRP suggest that the monoamine nuclei have an internal organization such that activity in subsets of dorsal raphe and locus coeruleus cells may independently influence separate populations of neurons within serotonergic and noradrenergic terminal fields of the neocortex. Moreover, double-labeling protocols have revealed single dorsal raphe neurons which project to both rat visual cortex and cerebellar paraflocculus, areas which are known to receive visual

information. The emphasis of the proposed studies will be to explore the possibility that central monoaminergic projections are organizaed according to the sensory function of target neuronal circuits and whether such an organization would be consistent with a postulated role of these systems in attentional mechanisms.

3. Status of Research

A brief description of individual studies (see also Publications Supported) conducted during the past year is provided below.

Norepinephrine and Serotonin Actions in Rat Visual Cortex: A report describing the effects of microiontophoretically applied NE and 5-HT on rat visual cortical neuron responses to moving visual stimuli was completed and accepted for publication (see item #4).

Norepinephrine and Serotonin Actions on Receptive Field Properties of Cat Visual Cortical Neurons: A recently initiated study has begun to examine the effects of norepinephrine (NE) and serotonin (5-HT) on receptive field properties (direction selectivity, orientation and velocity preference) of cat visual cortical neurons. A previous study in rat had demonstrated a modulatory influence of NE and 5-HT on visual cortical neuron responses to moving visual stimuli such that receptive field borders were more sharply defined by unit firing rates during NE administration and less distinct during 5-HT. These experiments in rat did not address the issue of monoamine effects on velocity and orientation tuning and direction selectivity. Preliminary results in cat indicate that NE can cause selective shifts in the velocity tuning curve as well as increase direction selectivity of individual neurons. Additional experiments are needed to complete this study, but the present results lead us to consider the possibility that monoamine-induced changes in primary sensory circuit function may alter sensory perception of behaviorally significant cues.

Pharmacological Characterization of NE Modulatory Interactions with Glutamate in Cells Recorded from Cortical Tissue Slices: A recently completed study (see item #5) has established that NE-induced potentiation of glutamate-evoked excitatory discharges in somatosensory neurons recorded from cortical tissue slices is mediated by activation of alpha type adrenoceptors. NE and alpha agonists are also capable of revealing robust excitatory discharges in response to otherwise subtthresholddoses of glutamate. Other results indicated that these potentiating actions of NE do not involve increased intracellular levels of cyclic AMP but may involve the actions of a C kinase. Overall, the results of this extracellular study

contradict previous findings of intracellular experiments in which beta agonists were found to mimic NE-induced changes in neuronal excitability. Moreover, data obtained from these intracellular studies is not adequate to explain the "gating" effect of NE on otherwise subthreshold doses of glutamate. For these reasons future studies will explore the interactions between NE and intracellular responses of cortical units to juxtathreshold stimulation of afferent synaptic pathways. The goal will be to define the mechanism(s) responsible for shifting the threshold of cortical neuron detection of incoming signals and for amplifying responses to already supra-threshold synaptic inputs.

Pharmacological Characterization of NE Facilitating Effects in Terms of Glutamate Receptor Subtypes: A preliminary set of studies has also begun to examine interactions between NE and NMDA-, kainate- and quisqualate-induced excitatory responses as a means of determining the specificity of noradrenergic modulatory actions at NMDA and non-NMDA receptor sites. Although NE can potentiate responses to both kainate and NMDA, the magnitude of this facilitatory effect is greater on NMDA induced responses. Further studies are needed to determine if this differential modulatory effect represents a potentially significant functional specificity for NMDA mediated responses.

Noradrenergic Influence on GABA-induced Membrane Conductance Changes: Another ongoing study using intracellular recording procedures in cortical tissue slices has demonstrated that NE can enhance GABA-induced membrane conductance changes. Such actions are mimicked and blocked by beta agonists and antagonists, respectively, and also mimicked by agents which elevate intracellular levels of cyclic AMP (eg. forskolin and 8-bromo-cyclic AMP). As such these noradrenergic influences on transmitter-induced conductance changes are consistent with previously observed effects of synaptically released and iontophoretically applied NE on extracellularly recorded responses of cortical and cerebellar neurons to GABA. Similar enhancement of membrane response to muscimol indicate that this effect is specific for the GABA A receptor. Overall, these data in conjunction with structural studies of the GABA receptor suggest that a cyclic AMP dependent phosphorylation of the receptor protein could be responsible for NE-induced changes in GABA efficacy. While many additional studies will be necessary to establish the validity of this hypothesis, the results decribed here provide the conceptual framework necessary for evaluating such a postulated mechanism.

Many of the cells in which such NE-induced membrane effects have been observed have been morphologically identified as layer V pyramidal neurons by intracellular staining with biocytin. By categorizing the cortical cell types which are sensitive to NE modulatory actions, we hope to be able to better understand the potential contribution of the noradrenergic system to signal

processing within neocortical microcircuits.

Psychostimulant Drug Studies: Two reports describing cocaine actions in the somatosensory cortex and cerebellum of anesthetized rats have been completed and submitted for publication in Brain Research. Overall, these results suggest a link between cocaine's psychostimulant effects on sensory awareness and cognitive function and the role of the noradrenergic system in arousal and selective attention.

Distribution of Monoamine Neurons Projecting to the Rat Somatosensory System: An ongoing study utilizing double retrograde labeling strategies is examining the intranuclear distribution of dorsal raphe (DR) and locus coeruleus (LC) neurons that send collateral projections to regions of the rat somatosensory systems which sequentially process the same sensory information. These somatosensory areas include the spinal nucleus of the trigeminal, VPM thalamus and barrelfield cortex each of which receives and relays afferent signals from the rat vibrissae. The results obtained to date indicate that overlapping zones within the LC nucleus contain cells which project to these somatosensory areas. Furthermore, approximately 15-20% of labeled LC neurons send axons to more than one relay station along the somatosensory pathway. An issue which has not yet been resolved is whether or not this apparent functional organization is unique for each of several different sensory modality systems. Nevertheless, the hypothesis which will be tested in additional experiments is that cells within the monoamine containing nuclei are organized according to functional properties of their efferent targets.

Comments on Progress: During the past year a number of previously initiated studies have been brought to completion and the results prepared for publication. In the coming year we will continue to focus considerable attention on the characterization of NE and to a lesser extent 5-HT effects on the membrane responses of identified cortical neurons to non-monoamine synaptic inputs and transmitter substances. The long term goal of these studies is to determine the sensitivity of specific classes of neocortical neurons to monoaminergic modulatory actions and develop a more comprehensive view of how synaptically released NE and 5-HT might influence the function of ensembles of functionally related cortical neurons. Other studies in the visual cortex should be capable of revealing the potential for synaptically released monoamines to regulate specific parameters of individual sensory neuron function. Aside from determining the lowest threshold modulatory action of NE and 5-HT in anesthetized and reduced preparations, a new phase of studies in awake unanesthetized animals will be directed toward a demonstration that synaptically released monoamines can modulate cortical neuronal circuit function under physiologic conditions.

4. Publications Supported

Papers:

Waterhouse, B.D., Azizi, S.A., Burne, R.A. and Woodward, D.J. 1990. Modulation of rat cortical area 17 neuronal responses to moving visual stimuli during norepinephrine and serotonin microiontophoresis. Brain Res. (In Press)

Waterhouse, B.D., Sessler, F.M., Liu, W. and Lin, C.-S. 1990. Second messenger mediated actions of norepinephrine on target neurons in central circuits: intracellular mechanisms and functional consequences. Prog. Brain Res. (In Press)

Mouradian, R.D., Sessler, F.M. and Waterhouse, B.D. 1990. Noradrenergic potentiation of excitatory transmitter action in cerebrocortical slices: evidence for mediation by alpha receptor linked activation of protein kinase C. Brain Res. (In Press)

Abstracts

Altman, D.W., Lin, C.-S. and Waterhouse, B.D. 1990. Distribution of locus coeruleus neurons that project to rat VB thalamus and barrelfield cortex. Soc. Neurosci. Abst.

McLean, J., Lin, C.-S. and Waterhouse, B.D. 1990. Effects of norepinephrine on velocity tuning and direction selectivity of rat visual cortical neurons. Soc. Neurosci. Abst.

Manuscripts

Jimenez-Rivera, C. and Waterhouse, B.D. Effects of systemically and locally-applied cocaine on cerebrocortical neuron responsiveness to thalamocortical inputs and glutamate. Brain Res. (Submitted)

Shin, H.-C., Jimenez-Rivera, C.A., Waterhouse, B.D. and Chapin, J.K. Cocaine-induced changes in sensory responsiveness of simultaneously recorded single neurons in the SI cortex of behaving rats. (In Preparation).

Waterhouse, B.D., Stowe, Z.N., Jimenez-Rivera, C.A., Woodward, D.J. and Sessler, F.M. Cocaine actions in central noradrenergic circuits: enhancement of cerebellar Purkinje neuron responses to iontophoretically applied GABA. Brain Res. (Submitted)

5. **Professional Personnel Directly Involved in AFOSR-87-0138**

Barry D. Waterhouse, Ph.D. (PI)
Assocate Professor of Physiology and Biophysics

Francis M. Sessler, Ph.D.
Research Assistant Professor

Judith McLean, Ph.D.
Research Instructor

Robert Mouradian, M.S.
Graduate Student

Weimin Liu, M.S.
Graduate Student

Daniel Altman
Graduate Student

6. **Coupling Activities**

Invited presentations:

"Regulation of $GABA_A$ Receptor Function in Brain: Is There a Role of Phosphorylation", Winter Conference on Brain Research-1990, Jan. 27-Feb. 3, Snowmass, CO.

"Mediation of Noradrenergic Modulatory Effects by Intracellular Signal Transduction Mechanisms", International Symposium on the Neurobiology of the Locus Coeruleus, May 16-19, Post Falls, ID.

7. **New Discoveries, Inventions, etc.**

N/A